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Toxicity of 1,1-Dichloroethylene (Vinylidene Chloride) to Aquatic Organisms

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Midland, MI

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16. ABSTRACT: Studies were conducted to determine the acute toxicity of 1,1-dichloroethylene [(vinylidene chloride) VDC] to fish and macroinvertebrates. The methods included a 96-hour static toxicity test using fathead minnows, Pimephales promelas Rafinesque; a 48-hour static toxicity test using water fleas, Daphnia magna Straus; and a 13-day flow-through toxicity test using the fathead minnow, Pimephales promelas Rafinesque. The 96-hour static LC50 value for fathead minnows was 169 (161 to 179)* mg/L. The LC50 value is the calculated concentration of toxicant which would kill 50 percent of the test organisms within a specified time period, e.g., 96 hours. The 48-hour static LC50 for daphnids was 11.6 (9.0 to 14.0) mg/L. The 96-hour flow-through LC50 value for fathead minnows was 108 (85 to 117) mg/L. The threshold LC50 value in flowing water was demonstrated after 7 days to be 29 (23 to 34) mg/L. The threshold LC50 value is achieved when there is no further decline in the LC50 value over a period of three to four days or more. Loss of body equilibrium (swimming disorientation) was the major sublethal toxic effect noted in the static and flow-through fish tests. Many fish affected in the static test recovered in 48 hours, perhaps because of volatilization of the toxicant. However, all affected fish in the flow-through test died by day 7. The 24- and 48-hour LC50 water flea test values were identical, probably because of volatilization of the test material during the first 24 hours. The difference in the static and flow-through fish toxicity values stresses the importance of conducting a flow-through test with volatile chemicals to adequately determine acute and longer term exposure effects (>96 hours).

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by

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FOREWORD

The Environmental Research Laboratory-Duluth is charged in part with the development of aquatic life criteria for environmental pollutants.

The data reported in this manuscript are the result of research conducted by the staff of the Environmental Sciences Research Laboratory of The Dow Chemical Company, Midland, Michigan 48640.

Norbert Jaworski, Ph.D
Director
Environmental Research Laboratory-Duluth

ABSTRACT

Studies were conducted to determine the acute toxicity of 1,1-dichloroethylene [(vinylidene chloride) VDC] to fish and macroinvertebrates. The methods included a 96-hour static toxicity test using fathead minnows, Pimephales promelas Rafinesque; a 48-hour static toxicity test using water fleas, Daphnia magna Straus; and a 13-day flow-through toxicity test using the fathead minnow, Pimephales promelas Rafinesque.

The 96-hour static LC50 value for fathead minnows was 169 (161 to 179)* mg/L. The LC50 value is the calculated concentration of toxicant which would kill 50 percent of the test organisms within a specified time period, e.g., 96 hours. The 48-hour static LC50 for daphnids was 11.6 (9.0 to 14.0) mg/L. The 96-hour flow-through LC50 value for fathead minnows was 108 (85 to 117) mg/L. The threshold LC50 value in flowing water was demonstrated after 7 days to be 29 (23 to 34) mg/L. The threshold LC50 value is achieved when there is no further decline in the LC50 value over a period of three to four days or more. Loss of body equilibrium (swimming disorientation) was the major sublethal toxic effect noted in the static and flow-through fish tests. Many fish affected in the static test recovered in 48 hours, perhaps because of volatilization of the toxicant. However, all affected fish in the flow-through test died by day 7. The 24- and 48-hour LC50 water flea test values were identical, probably because of volatilization of the test material during the first 24 hours.

The difference in the static and flow-through fish toxicity values stresses the importance of conducting a flow-through test with volatile chemicals to adequately determine acute and longer term exposure effects (>96 hours).

This report was submitted by The Dow Chemical Company in cooperation with the U.S. Environmental Protection Agency, Duluth. This report covers the period from October 12, 1976, to February 13, 1977, and work was completed on October 13, 1977.

*95 percent confidence interval in parentheses

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SECTION 1

INTRODUCTION

1,1-Dichloroethylene [vinylidene chloride (VDC)] is a raw material which is polymerized in the production of resins and latexes. It is also a raw material in other chemical processes, such as copolymerization with vinyl chloride in the production of SARAN* plastic films. Because of VDC's widespread use, large production, and bulk transport, certain basic environmental tests were considered necessary.

These environmental tests included static acute toxicity tests using fathead minnows, Pimephales promelas Rafinesque, and water fleas, Daphnia magna Straus. An acute flow-through fish toxicity test was also run with fathead minnows, Pimephales promelas Rafinesque, to determine the differences in results between the static and flow-through test systems.

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SECTION 2

METHODS AND MATERIALS

CHEMICAL

The test chemical used in the static fathead minnow and water flea toxicity tests and the flow-through fathead minnow toxicity test was the distilled monomer with a minimum of 99.5 percent VDC. The major impurities were identified as the cis and trans isomers of 1,2-dichloroethylene. Some physical properties of VDC are presented in Table 1.

TABLE 1. PHYSICAL PROPERTIES OF VDC

Specific gravity[1]	1.202 to 1.212
Melting point[1]	-122.5°C
Boiling point[1]	31.7°C
Flash point[1]	-15°C
Vapor pressure[2]	409 mmHg @ 15°C
Water solubility[2]	0.26 wt percent @ 15°C

[1]The Merck Index. M. Windholz, editor, Merck and Co., 9th Edition, 1976.

[2]DeLassus, P. T. 1977. Solubilities of Vinyl Chloride and Vinylidene Chloride in Water. SCP-106. The Dow Chemical Company. Midland, Michigan 48640.

DILUTION WATER

The dilution water used in the fathead minnow and water flea toxicity tests was carbon filtered raw Lake Huron water. Raw Lake Huron water was obtained from the city of Midland, Michigan's, water pipeline prior to treatment for the city's water supply. This water exhibits chemical characteristics listed in Tables 2 and 3 (Hunemorder *et al.*, 1977).

TABLE 2. LAKE HURON WATER ANALYSES

Dissolved oxygen[a]	>80 percent saturated
pH[b]	7.9
Total alkalinity, mg/L as $CaCO_3$	85
Total hardness, mg/L as $CaCO_3$	100
Specific conductivity,[c] µmhos/cm	170

[a]Yellow Springs Instruments Model 54 - Oxygen Meter.

[b]Sargent-Welch pH Meter - Model LS.

[c]Yellow Springs Instruments Model 31 - Conductivity Bridge.

TABLE 3. RAW LAKE HURON WATER ANALYSES PRIOR TO CARBON FILTRATION

Parameter	Value (mg/L)
Alkyl benzene sulfonate	(0.10)*
Arsenic	<0.005
Barium	0.011
Cadmium	(0.01)*
Chlorine	10.0
Chromium	(0.01)*
Copper	0.03
Cyanide	(0.01)*
Fluoride	<0.5
Iron	0.1
Lead	(0.03)*
Magnesium	7.0
Manganese	0.01
Nitrate	0.5
Phenols	(0.001)*
Selenium	(0.02)*
Silver	(0.01)*
Sulfate	16
Total dissolved solids	144
Zinc	0.03
PCB's	$<0.02 \times 10^{-3}$
Mercury	(0.002)*

*Parameter was below the limits of detection which are included in parentheses.

FISH

Adult fathead minnows, Pimephales promelas Rafinesque, were used in the fish toxicity tests. They were purchased from White Bear Bait Company, White Bear Lake, MN, and transported by airfreight to our laboratory. The fathead minnows were held in dilution water at 12°C ± 1°C for at least 10 days prior to testing. They were kept in a 16-hour light/8-hour dark cycle. A synthetic diet (Mehrle, 1976) was fed to all fish during the acclimation period. Feeding was stopped 3 days prior to the tests to empty the digestive tract.

WATER FLEAS

First instar Daphnia magna Straus were used in the static water flea test. First instar water fleas were defined as being less than 24 hours old. Stock cultures of the test species were maintained in 18L glass aquaria at 17°C ± 1°C, with a 16-hour light/8-hour dark cycle. Cultures were fed a suspension of finely ground Master Mix Trout Pellets (Master Mix Feeds, Portland, Michigan) and alfalfa (10 mg solids per mL of suspension). First instar water fleas were collected by pouring stock culture water through three nested baskets made of stainless steel mesh. A 16-mesh screen basket allowed first instars to pass through while retaining the larger water fleas. A 25-mesh screen retained intermediate sizes, but allowed first instars to pass through. A 50-mesh screen retained the first instars.

SECTION 3

EXPERIMENTAL PROCEDURES

STATIC WATER FISH TOXICITY TEST

The static water fish toxicity test was conducted according to test methods described by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Methyl alcohol (glass distilled) was used as the carrier solvent to prepare stock solutions of VDC. A control containing the same amount of alcohol present in the highest chemical concentration and a Lake Huron water control were included in each test series. The fish were not fed nor were test solutions renewed during the test. Dead or affected fish were counted daily and the dead fish removed.

The toxicity test was conducted by placing 10 liters of 12°C dechlorinated Lake Huron water in a round all-glass aquarium measuring 26 cm deep by 24.5 cm in diameter, having a maximum capacity of 12 liters. VDC stock solution was added by pipette below the water surface and swirled quickly to disperse it. Ten fish, averaging 35 mm standard length and 0.8 gm, were added to each aquaria. The aquaria were covered with SARAN* plastic food wrap to retard volatilization during the first 24 hours. The loading of the aquarium was 0.8 gram fish per liter. A constant temperature water bath kept the aquaria temperature at 12°C $\pm$ 1°C.

The nominal concentration was used to calculate LC50 toxicity value. The nominal concentration is the value calculated from the amount of VDC initially added to a volume of water. However, the actual concentration of VDC in each aquaria was probably less than nominal concentration because of losses from volatilization. The highest concentration of methanol used in any test solution did not exceed 0.5 mg/L. Dissolved oxygen (DO) was monitored on days 1 and 3 and did not drop below 60 percent saturation. The test was terminated after 96 hours.

STATIC WATER FLEA TOXICITY TEST

The static water flea test exposed Daphnia magna Straus, reared in our laboratory, to various concentrations of VDC in dilution water at 17°C for 48 hours. Stock solutions of VDC were prepared in methanol both to dilute the test material and to facilitate rapid mixing with water. The

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required amount of stock solution was combined with sufficient dilution water to make a final volume of 200 mL in a 250 mL glass test beaker. A water and a solvent control were also set. The solvent control contained the same amount of methanol as the highest test chemical concentration. Methanol concentrations did not exceed 0.1 mg/L. Ten first instar water fleas were added to each beaker and the beakers set in a constant temperature incubator having a 16-hour light/8-hour dark cycle. There were three beakers for each exposure concentration and each control. Mortality data were recorded at 24 and 48 hours; death was defined as no response to a gentle prodding. Dead organisms were not removed from any test beaker during the test.

FLOW-THROUGH TEST

In the flow-through test, one liter of fresh solution containing VDC was supplied every seven minutes to each exposure aquaria throughout the testing period. For each cycle, a methanol stock solution (containing 626 mg of VDC per ml) was delivered by pump (Harvard Apparatus 1302 Lambda) to a covered mixing chamber to give a nominal exposure level of 400 mg/L in the first exposure aquaria. A flow-through dilutor system similar to that described by Mount and Brungs (1967), was used to deliver the various VDC concentrations. The aquaria were molded glass, measuring 18.5 cm wide and 28.5 cm long. The water depth was 12.5 cm, giving 6.6 liters water per aquarium. A clear plastic cover was placed over each exposure aquarium to retard volatilization of title material. VDC concentrations were determined by GC analysis, and proper dilutor operation confirmed before the fish were transferred into the aquaria. The dilutor was designed to supply a series of nominal concentrations each 75 percent of the preceding value. The loading per aquaria was 0.09 gm fish/liter/day.

The concentration of VDC in each test chamber was monitored by gas chromatography. Twenty mL samples of each aquaria were taken once per day for the first five days (Monday through Friday) and then on Monday, Wednesday, and Friday for the remainder of the test. Samples were taken in glass vials with perfluorocarbon plastic-lined caps. Samples were immediately put on ice and analyzed by gas chromatography.

The fish were not fed during the first 96 hours of the test. However, from day 5 to 13, the fish were fed a synthetic diet once daily. The flow-through test was terminated when the toxicity curve (LC50 vs. exposure time) became stable.

VDC ANALYSIS

Concentrations of 1,1-dichloroethylene were monitored using a HP 5700 Gas Chromatograph[a] (GC) with a flame ionization detector. The parameters for the GC analysis were:

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[a]Hewlett-Packard Corp., Avondale, PA 19311

1/4" OD x 2 mm ID glass column
1 µl on column injection
0.4 percent E1500 on Carbopack A
60°C isothermal
150°C detector temperature Flame Ionization Detector
20 cc/minute carrier gas N_2
1 x 1 attenuation

The GC was calibrated daily using a fresh standard solution prior to analysis of test samples. To prepare the working standard, an aliquot of the VDC concentration standard in methyl alcohol was diluted with water. Direct aqueous injection was used for the water samples. The title compound was qualitatively identified by comparison with the retention time of the standard. Quantitation of VDC concentration was by comparison of peak areas of the samples to that of a standard solution using the external standard program of the Hewlett-Packard 3380 integrator.

SECTION 4

STATISTICAL CALCULATIONS

Lethal Concentration (LC) results for the flow-through fish test were calculated in terms of the average analyzed concentration producing death to 10 percent, 50 percent, and 90 percent of the test organisms (LC10, LC50, and LC90) after exposure for a specified period of time. The 95 percent confidence interval was calculated for each LC value. The static water flea and static fish test LC50 values were calculated using nominal VDC water concentrations. A computer program of Finney's methods of probit analysis (Finney, 1952) was utilized to calculate the LC values, the confidence interval, and the slope of the regression curve for the static water flea test and the flow-through fish test. The LC50 values and 95 percent confidence intervals for the 96-hour static fish toxicity test were calculated using a computer program of Thompson's methods of moving averages (Thompson, 1947).

Effect Concentration (EC) results for the flowing water fish test were calculated as the average analyzed concentration which produced an observed adverse effect in 50 percent of the test organisms exposed for a specified time period. Adverse effects observed included loss of body equilibrium, melanization, and mortality. Effect concentration values were calculated using Finney's method of probit analysis.

SECTION 5

RESULTS AND DISCUSSION

The 96-hour static fish toxicity LC50 value was 169 mg/L (161 to 179). Data are presented in Table 4. The 48-hour static water flea toxicity LC50 value was 11.6 mg/L (9 to 14). The 24-hour and 48-hour water flea LC values are identical, indicating that the compound probably had volatilized from the exposure beakers. Table 5 gives a more detailed summary of the calculated water flea LC values.

The calculated LC values for the 13-day flow-through fish toxicity test are given in Table 6. The exposure concentrations used in the LC calculations were the average of 8 GC measured values per concentration. A summary of the GC measured concentrations over the duration of the test are presented in Table 7. Day to day variation in any one concentration was ± 13 percent of its average concentration. A computer drawn plot of LC50 values and their 95 percent confidence interval as a function of time (Figure 1) shows that the LC50 concentration remained steady through day 4, took a steep drop from day 5 to 6, then stabilized at 29 mg/L (25 to 34) on day 7 where it remained until the test ended.

TABLE 4. VDC STATIC ACUTE FISH TOXICITY LC50 VALUES

Nominal Concentration (mg/L)

24 h		48 h		96 h	
LC50	95 percent CI[a]	LC50	95 percent CI	LC50	95 percent CI
175	167 to 186	169	161 to 179	169	161 to 170

[a]Confidence interval.

TABLE 5. VDC STATIC ACUTE WATER FLEA LC VALUES

Hours	Nominal Concentration (mg/L) LC10	LC50	LC90	Slope[a]
24	3.8 (1.3 to 5.8)[b]	11.6 (9.0 to 14.0)	35.9 (25.2 to 87.8)	2.6 (1.4 to 3.8)
48	3.8 (1.3 to 5.8)	11.6 (9.0 to 14.0)	35.9 (25.2 to 87.8)	2.6 (1.4 to 3.8)

[a]Slope represents the rate of change in mortality as a function of time on a logarithmic scale.

[b]95 percent confidence interval in parentheses.

TABLE 6. VDC FLOW-THROUGH TOXICITY TEST LC VALUES AND 95 PERCENT CONFIDENCE INTERVALS USING FATHEAD MINNOWS, PIMEPHALES PROMELAS

Day	Measured Concentration (mg/L)			
	LC10	LC50	LC90	Slope[a]
1	94 (27 to 106)[b]	116 (99 to 143)	142 (125 to 555)	14.3
2	93 (38 to 103)	108 (85 to 117)	126 (116 to 221)	19.3
3	93 (38 to 103)	108 (85 to 117)	126 (116 to 221)	19.3
4	93 (38 to 103)	108 (85 to 117)	126 (116 to 221)	19.3
5	67 (43 to 79)	97 (82 to 115)	140 (118 to 226)	7.9
6	54 (38 to 63)	74 (63 to 85)	101 (87 to 138)	9.3
7	20 (14 to 24)	29 (25 to 34)	43 (36 to 62)	7.7
8	20 (14 to 24)	29 (25 to 34)	43 (36 to 62)	7.7
9	20 (14 to 24)	29 (25 to 34)	43 (36 to 62)	7.7
10	20 (14 to 24)	29 (25 to 34)	43 (36 to 62)	7.7
11	20 (14 to 24)	29 (25 to 34)	43 (36 to 62)	7.7
12	20 (14 to 24)	29 (25 to 34)	43 (36 to 62)	7.7
13	20 (14 to 24)	29 (25 to 34)	43 (36 to 62)	7.7

[a]Slope represents the rate of change in mortality as a function of time on a logarithmic scale.

[b]95 percent confidence interval in parentheses below corresponding LC value.

TABLE 7. MEASURED VDC WATER CONCENTRATIONS* FROM THE FLOW-THROUGH FISH TOXICITY TEST USING FATHEAD MINNOWS, PIMEPHALES PROMELAS

	Nominal Concentration (mg/L)								
	400	300	225	170	125	95	70	50	40
Measured Concentration (mg/L)									
Day 0	140	131	88	59	47	39	24	19	22
Day 1	123	109	69	56	35	33	21	15	15
Day 2	100	87	68	41	27	24	18	13	13
Day 3	117	114	80	54	30	32	19	15	14
Day 4	125	107	71	56	42	31	22	16	16
Day 7	129	92	63	49	31	24	19	15	15
Day 9	150	109	73	55	43	35	21	19	16
Day 11	120	106	72	53	31	30	18	16	15
Average Measured Concentration	126	107	73	53	36	31	20	16	16

*Analyzed using a HP5700 GC with flame ionization detector.

FIGURE 1. DATA PLOT OF LC50 VALUES AND THEIR 95 PERCENT CONFIDENCE INTERVALS FOR THE VDC FLOW-THROUGH TEST

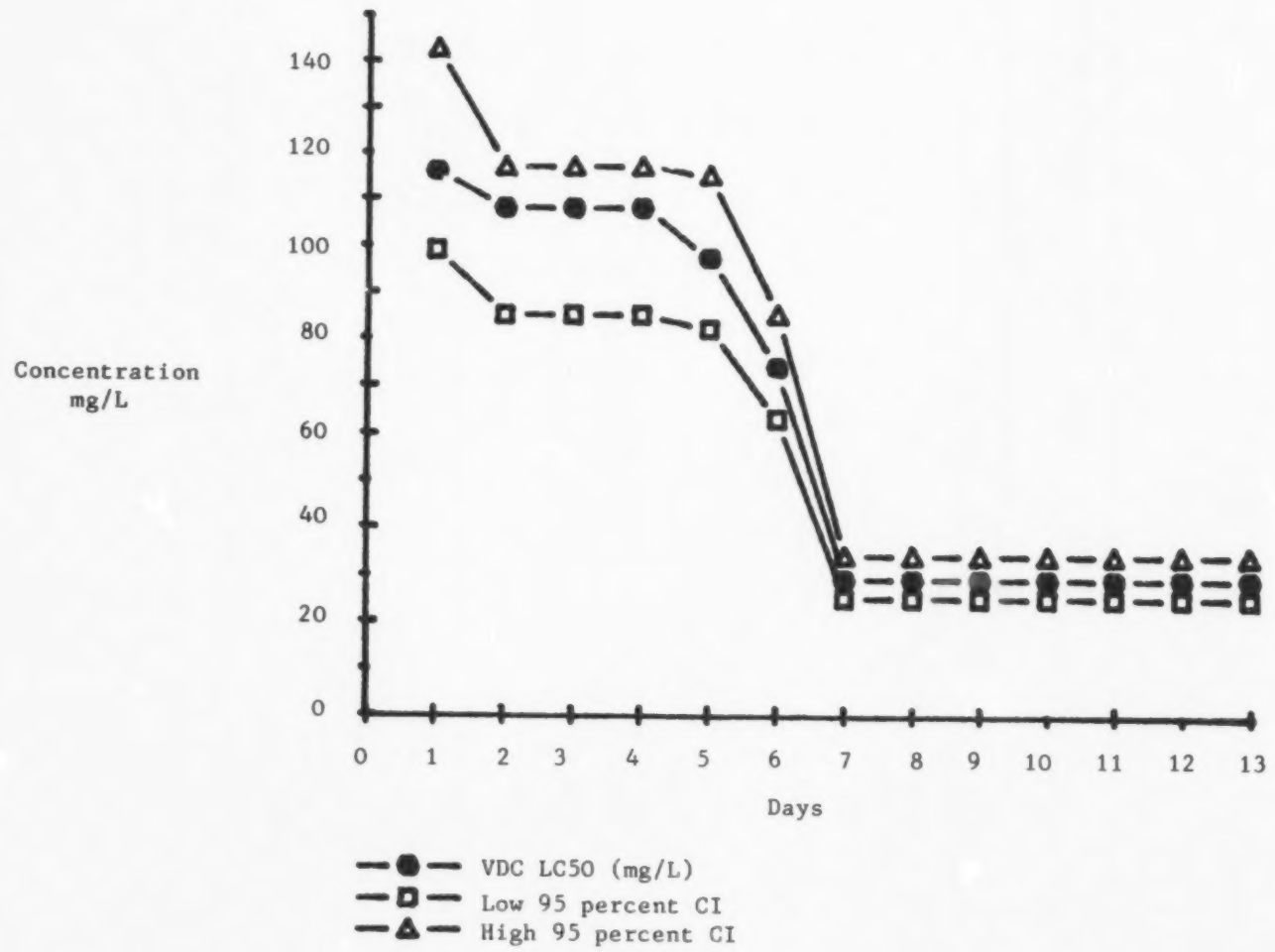


Table 8 compares the 24- through 96-hour LC50 values of both the static and flow-through toxicity tests. One might conclude from these mortality data there was little difference in the two systems, other than the approximate 35 percent difference in LC50 values. However, there were major differences in the observed distress effects between the two tests. In the static test, fish which were in distress (mainly affected with loss of equilibrium-swimming disorientation) during the first 24 hours, had either died or completely recovered in 48 hours, exhibiting no further distress symptoms nor any mortality between 48 and 96 hours. The 96-hour mortality effects were very similar in both tests. However, in the flow-through test, distressed fish continued to show symptoms (again a loss of body equilibrium) from 48 to 96 hours and never recovered. All distressed fish were dead by day 7 of the flow-through test. The data plot of EC50 vs. LC50 values given in Figure 2 shows this phenomenon.

The LC50 value differences in the static and flow-through tests were probably due to the high volatility of the VDC. In the static test, the distressed fish were observed to recover in 48 hours, and no further increase in mortality occurred. However, in the flow-through test where the concentration of VDC was kept at a constant level, the fish continued to become distressed and die through day 7. Thus, the flow-through test, which provided a chronic exposure of toxicant to the test organism, was able to provide additional valuable data about the toxicity of the volatile title compound.

TABLE 8. COMPARISON OF FLOW-THROUGH AND STATIC FISH TOXICITY LC50 VALUES FOR VDC EXPOSED FATHEAD MINNOWS, PIMEPHALES PROMELAS

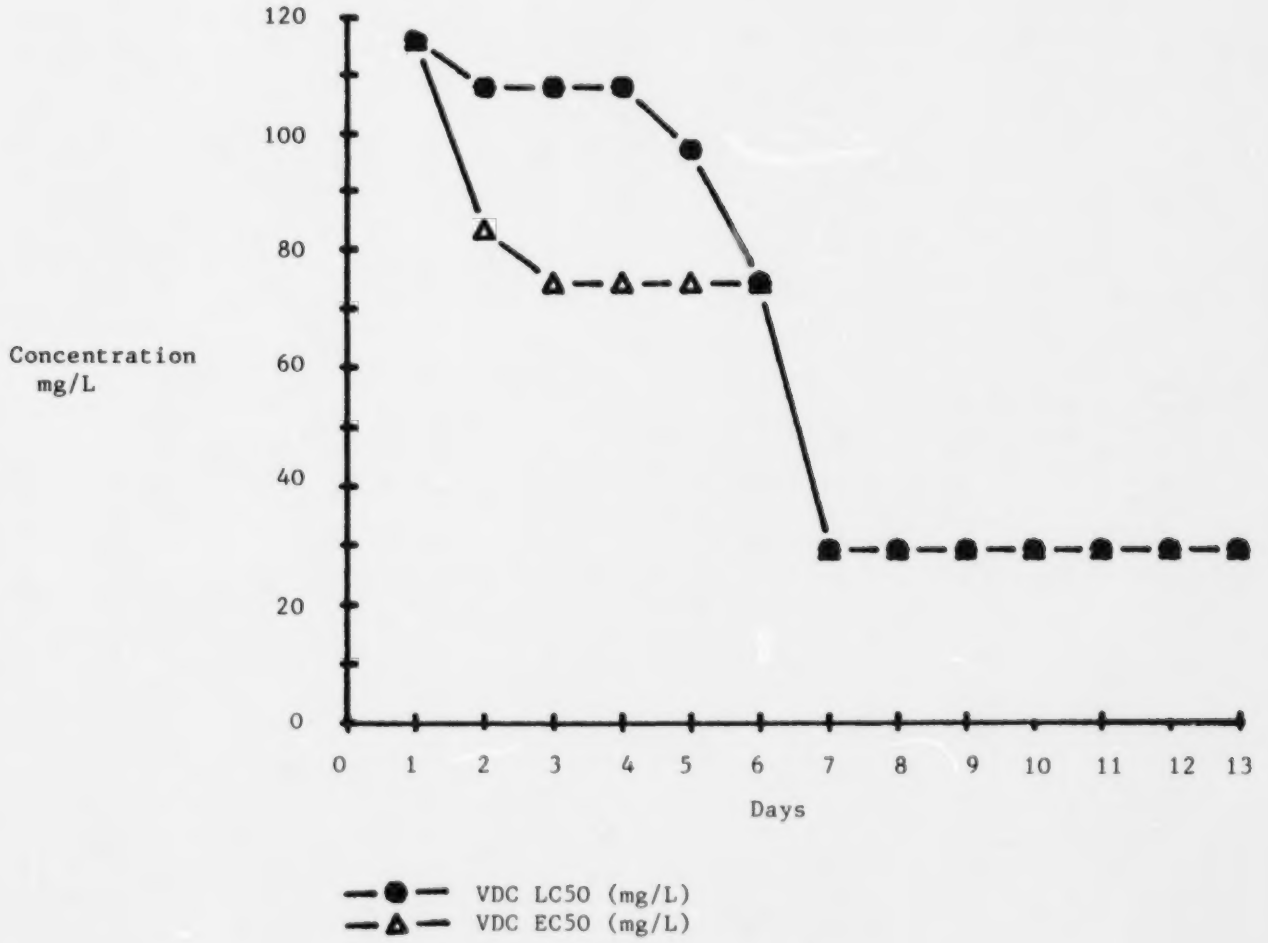
	Concentration (mg/L)	
Hour	Flow-through[a]	Static[b]
24	116 (99 to 143)[c]	157 (167 to 186)
48	108 (85 to 117)	169 (161 to 179)
72	108 (85 to 117)	169 (161 to 179)
96	108 (85 to 117)	169 (161 to 179)

[a]Calculated using the daily measured concentrations averaged over the 13 day test.

[b]Calculated using the nominal concentration (amount of VDC added at start of test).

[c]95 percent confidence interval in parentheses.

FIGURE 2. DATA PLOT OF THE LC50 AND EQUILIBRIUM EC50 VALUES FOR THE VDC FLOW-THROUGH TEST



REFERENCES

Committee on Methods for Toxicity Tests with Aquatic Organisms. "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians." EPA-660/3-75-009, U.S. Environmental Protection Agency, Corvalis, Oregon, 1975. 67 pp.

Finney, D. J. "Statistical Methods in Biological Assay. Cambridge University Press," London, 1952. 333 pp.

Hunemorder, E. J. et al. Dow Report ML AL 77-50645. Analytical Laboratories, Dow Chemical U.S.A., Midland, Michigan, 1977. 3 pp.

Mehrle, P. M. USDI Fish and Wildlife Services, Columbia, Missouri. Personal communication to E. A. Bartlett, Dow Chemical U.S.A., Midland, Michigan, 1976.

Mount, D. I., and W. A. Brungs. "A Simplified Dosing Apparatus for Fish Toxicity Studies." Water Research 1:21-29, 1967.

Thompson, W. R. "Use of Moving Averages and Interpolation to Estimate Median-Effective Dose." Bacteriological Reviews 11:(2) 115-143, 1947.